Local Antibiotic Delivery Using Tailorable Chitosan Sponges: The Future of Infection Control?

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Objectives: Local antibiotic delivery is a viable and attractive option for preventing infection. Unfortunately, the current options are limited and often necessitate surgical removal. This study evaluates the ability of a biodegradable and biocompatible chitosan sponge to minimize infection by delivering local antibiotics within the wound.

Methods: A complex musculoskeletal wound was created on the hindlimb of goats and contaminated with *Pseudomonas aeruginosa* (lux) or *Staphylococcus aureus* (lux) bacteria. These bacteria are genetically engineered to emit photons, allowing for quantification with a photon-counting camera system. The wounds were closed and similarly débrided and irrigated with 9 L normal saline using bulb-syringe irrigation 6 hours after inoculation. Goats were assigned to different treatment groups: a control group with no adjunctive treatment and an experimental group using a chitosan sponge loaded with either amikacin (for wounds contaminated with *P. aeruginosa*) or vancomycin (for wounds contaminated with *S. aureus*). The wounds were closed after the procedure and evaluated 48 hours after initial contamination. Serum levels of the antibiotics were also measured at 6, 12, 24, 36, and 42 hours after treatment was initiated.

Results: The wounds treated with the antibiotic-loaded chitosan sponge had significantly less bacteria than the untreated wounds ($P < 0.05$). The highest serum levels were 6 hours after treatment but remained less than 15% of target serum levels for systemic treatment. At study end point, all sponges were between 60% and 100% degraded.

Conclusions: The chitosan sponges are effective delivering the antibiotic and reducing the bacteria within the wounds.

Key Words: open fracture, infection, contamination, antibiotics, local delivery

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INTRODUCTION

Local antibiotic delivery is a viable and attractive treatment option for minimizing infection in complex musculoskeletal wounds. Several different techniques are used clinically that offer the advantage of delivery of high local antibiotic concentrations while maintaining low systemic levels.[1] Unfortunately, the current options, especially antibiotic-loaded polymethylmethacrylate (PMMA) beads, are limited and often necessitate surgical removal.[2,3] The ideal local antibiotic delivery vehicle would be a biocompatible substance with tunable elution and degradation rates that can be easily loaded at the point of care.

Chitosan, a natural polysaccharide made from shellfish sources such as crabs and shrimp, offers unique advantages in the treatment of open wounds. This polymer has inherent properties that promote wound healing[4,5] and when used as a vehicle for drug delivery, can be tailored to have predictable drug elution rates and biodegradation patterns.[6,7] The purpose of this study was to evaluate the ability of a biodegradable and biocompatible chitosan sponge to reduce infection in a contaminated musculoskeletal wound animal model through local delivery of antibiotics. We hypothesized that the amount of bacteria in wounds treated with the antibiotic-loaded chitosan sponge would be significantly less when compared with controls receiving only standard débridement and irrigation.

MATERIALS AND METHODS

This study has been conducted in compliance with the Animal Welfare Act, the Implementing Animal Welfare Regulations, and in accordance with the principles of the Guide for the Care and Use of Laboratory Animals. All procedures were performed in a laboratory accredited by the Association and Accreditation of Laboratory Animal Care following protocol approval from our Institutional Animal Care and Use Committee.

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Study I

After adequate anesthesia, using both preoperative epidural injection and general endotracheal anesthetic, a complex musculoskeletal wound was created on the proximal left hindlimb of 10 goats involving injury to all musculoskeletal tissues, which has been described previously.[8–11] This technique resulted in a reproducible complex musculoskeletal wound intended to mimic an open fracture without the need for skeletal stabilization.

After creation of the wound, it was contaminated with 1 mL of greater than 10^8 colony-forming units/mL *Pseudamonas aeruginosa* (lux),[8] which was spread evenly over the wound surface. These bacteria are genetically engineered to emit photons, allowing for quantification with a photon-counting camera system. Finally, the wounds were stapled closed and a sterile dressing was applied over the incision.

After surgery, the goats were recovered in their pens and allowed activity ad libitum for 6 hours. They were observed to ensure that all were ambulatory before additional procedures. The goats were again anesthetized and placed supine on the operating table in a custom, light-sensitive imaging chamber. A photon counting camera was used to capture the quantitative and spatial distribution of the bacteria within the wound.[8–11] After collection of baseline luminescent data, standard débridement and irrigation was performed with 9 L of normal saline using bulb-syringe irrigation. The imaging sequence was then repeated to obtain postdébridement and irrigation data.

Goats were then assigned to different groups: a control group with no adjunctive treatment and an experimental group using a chitosan sponge loaded with amikacin (Fig. 1). The chitosan sponges were prepared using a method previously described.[6,7] The freeze-dried chitosan sponges, which were approximately 4 cm in diameter, were removed from sterile peel packs and placed in a sterile dish on the back table. They were hydrated with the antibiotic solution (6.5 mL of solution containing 5 mg/mL of antibiotic yielding approximately 32.5 mg of available antibiotic) immediately before placement in the center of the wound. The antibiotic solution remaining in the dish was measured to determine the available antibiotic absorbed by the chitosan sponge. All wounds were closed with skin staples.

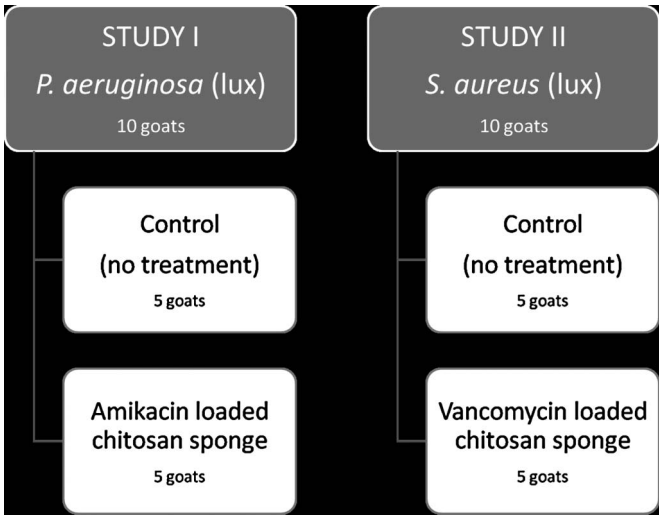

FIGURE 1. Ten goats were used in both Study I and Study II with five goats in both the control group (no treatment) and experimental group (antibiotic-loaded chitosan sponge).

The goats were recovered in their pens and allowed activity ad libitum. Serum levels of the antibiotics were measured at 6, 12, 24, 36, and 42 hours after treatment was initiated using a fluorescence polarization immunoassay instrument (TDxFLx; Abbott Laboratories, Abbott Park, IL). The goats were euthanized 48 hours after initial wound creation with an overdose of Fatal Plus (Vortech Pharmaceuticals, Dearborn, MI) and the bacteria were quantified using the imaging system. Wound characteristics as well as the amount of chitosan sponge remaining in the wound were recorded.

Study II

The same procedural steps were performed for wound creation, débridement and irrigation, and acquisition of luminescent data as in Study I, except that the goats (10) were contaminated with 1 mL of greater than 10^8 colony-forming units/mL *Staphylococcus aureus* (lux) (Xenogen 29; Caliper Life Science, Hopkinton, MA) instead of *P. aeruginosa* (lux). In Study I, we noticed that the sponge did not dissolve completely and decided to cut the chitosan sponge into quarters for Study II before loading with vancomycin (6.5 mL of solution containing 5 mg/mL of antibiotic) in an effort to hasten degradation (Fig. 2).

Retrieved Sponge Analysis/Elution Testing

Retrieved sponges were subjected to elution tests and antibiotic concentration was measured using fluorescence polarization immunoassay and normalized based on initial mass of sponge fragments.

Statistical Analysis

The postdébridement and 48-hour photon counts were normalized by the baseline (predébridement) counts. These ratios were analyzed for the treatment groups using an analysis of variance allowing for treatment. Statistical significance was set at a P value ≤ 0.05. All the values were reported as the mean $\pm$ the standard error of the mean.

RESULTS

The antibiotic-loaded chitosan sponge significantly reduced the bacteria in the contaminated wounds in both Study I and Study II ($P < 0.05$). One goat in the vancomycin treatment group died before completion of the study as a result of pneumonia and was excluded from data analysis. None of the other animals displayed any signs of illness or systemic sepsis.

Study I

All wounds contaminated with *P. aeruginosa* in both the control and treatment groups had similar reductions in bacterial levels after the 6-hour débridement and irrigation (19% $\pm$ 7% and 13% $\pm$ 2% of baseline, respectively). At 48 hours postinjury, the control group rebounded to 82% $\pm$ 35% of the 6-hour pretreatment bacterial levels.

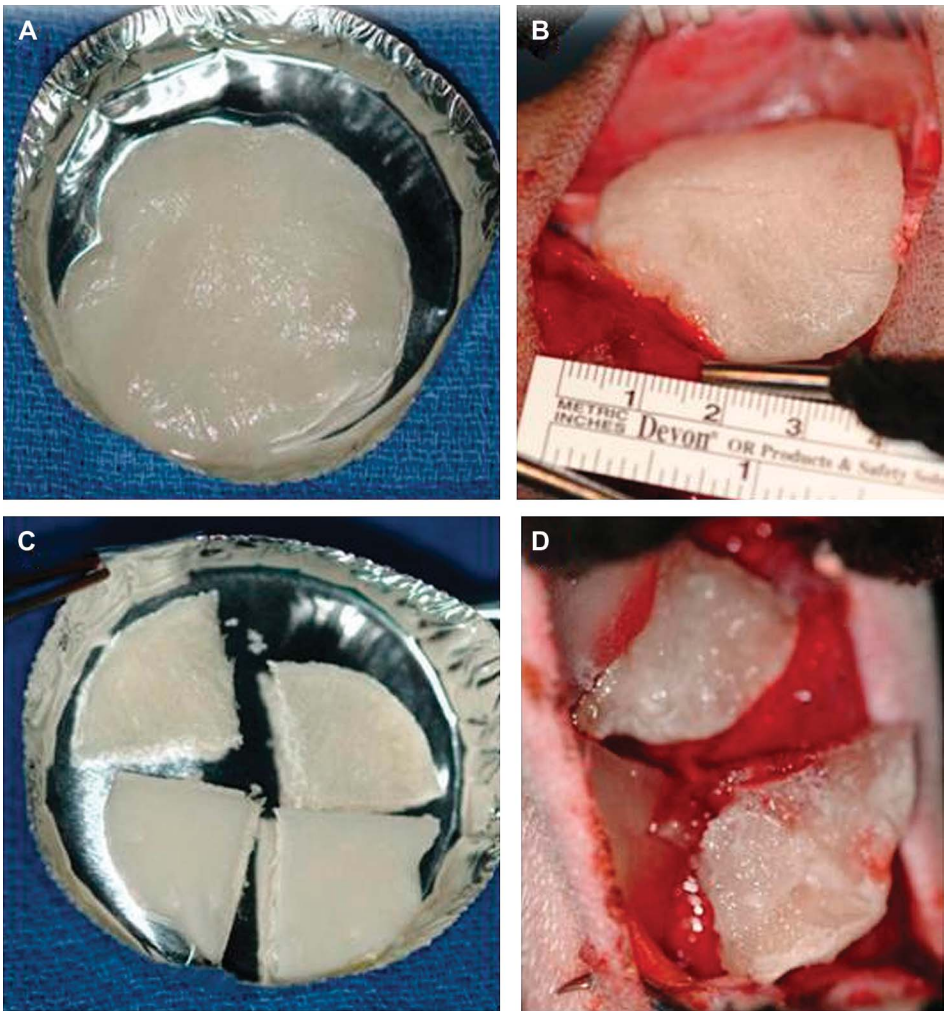

FIGURE 2. The amikacin-loaded chitosan sponge (A) was placed whole within the wound (B). The vancomycin-loaded chitosan sponge was cut into quarters (C) before placing within the wound (D).

All five goats treated with amikacin-loaded chitosan sponges had reduced bacterial levels to less than or equal to 1% (0.5% ± 0.3%) of the 6-hour pretreatment bacteria levels ($P < 0.05$) (Figs. 3 and 4).

Study II

All wounds contaminated with *S. aureus* in both the control and treatment groups also had similar reductions in bacterial levels after the 6-hour débridement and irrigation (38% ± 11% and 21% ± 4% of baseline, respectively). At 48 hours postinjury, the control group rebounded to an average of 677% ± 448% of the 6-hour pretreatment bacterial levels. Vancomycin-loaded chitosan sponges had significantly reduced bacterial levels to 18% ± 15% of the 6-hour pretreatment bacteria levels ($P < 0.05$) (Figs. 5 and 6).

Serum Antibiotic Concentration

The highest serum antibiotic levels were 6 hours after treatment initiation (0.53 ± 0.48 μg/mL for amikacin and 1.63 ± 0.65 μg/mL for vancomycin) but remained less than 15% of target serum levels for systemic treatment.

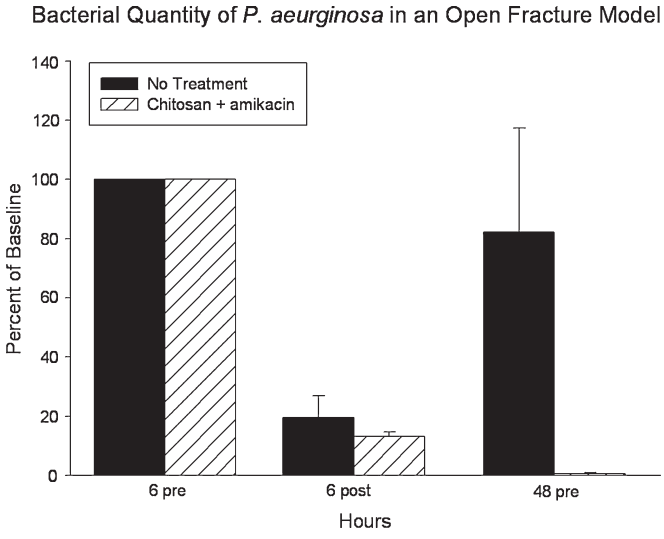

FIGURE 3. Results of Study I: Bacterial quanity in wounds contaminated with *Pseudomonas aeruginosa* (lux).

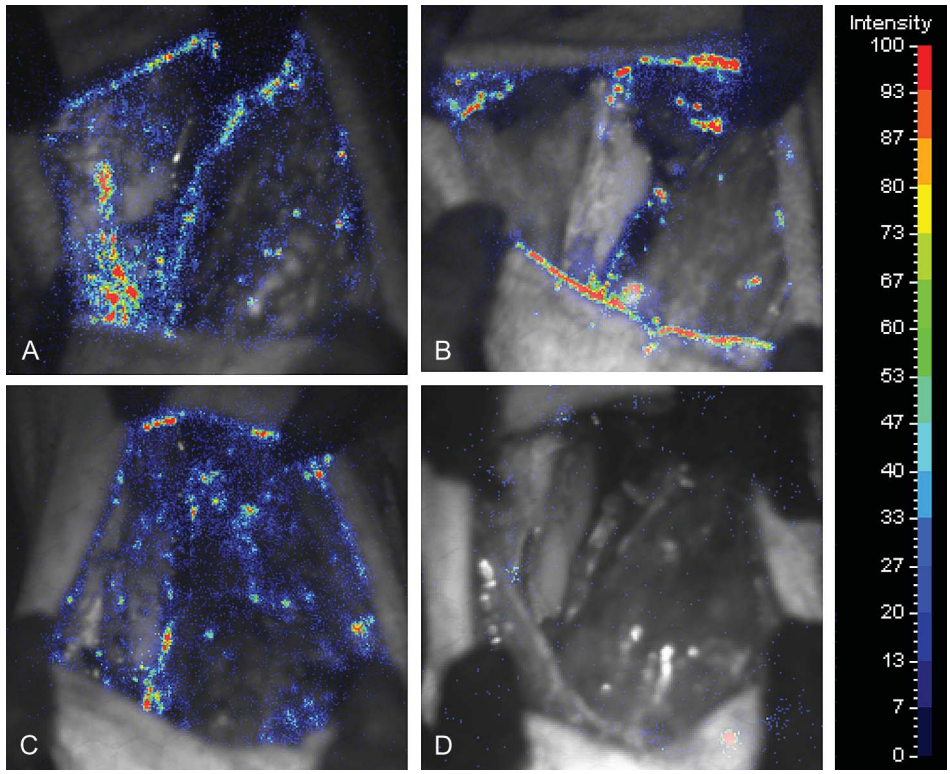

FIGURE 4. Representative imaging for Study I (wounds contaminated with *Pseudomonas aeruginosa* [lux]): Control group images are shown on the top with the 6-hour predébridement and irrigation image (A) and 48-hour image (B). The amikacin-loaded chitosan sponge group is show on the bottom with the 6-hour predébridement and irrigation image (C) and 48-hour image (D).

Chitosan Sponge Degradation

At study end point, the amikacin (n = 5) and vancomycin (n = 4) -loaded chitosan sponges were 87% ± 13% and 86% ± 3% degraded, respectively. Antibiotic remained detectable within all recovered sponges during elution tests using fluorescence polarization immunoassay at 12 hours after removal from the wound.

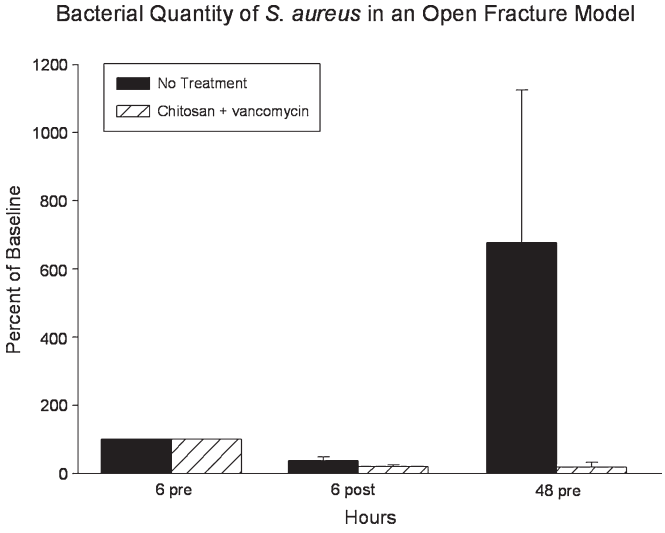

FIGURE 5. Results of Study II: Bacterial quanity in wounds contaminated with *Staphylococcus aureus* (lux).

DISCUSSION

An established large-animal contaminated musculoskeletal wound model was used to evaluate the effectiveness of a novel local antibiotic delivery vehicle in minimizing infection.[8–11] The chitosan sponge used in this study was specifically tailored to release antibiotics and dissolve over a period of 48 to 72 hours, which correlates to the typical time between subsequent débridement and irrigations for contaminated wounds.[12,13] As demonstrated in our study, when loaded with antibiotics, the chitosan sponges significantly reduced the bacterial load in contaminated musculoskeletal wounds after 42 hours of treatment ($P < 0.05$).

The majority of the chitosan sponges were more than 85% dissolved at 42 hours after placement in the wounds. In addition, consistent with other local drug delivery devices, the systemic antibiotic levels remained low at all time points, in most instances being below the detectable levels.[11] Changing the size of the sponge did not appear to affect the degradation rate.

Open fractures typically result from high-energy trauma and can cause significant soft tissue and skeletal injury. In these cases, systemic antibiotics may not be able to effectively penetrate the site of infection to deliver antibiotic levels above the minimum inhibitory concentration. Local antibiotic delivery eliminates this problem by providing high doses of antibiotics directly to the wound bed, well above the minimum inhibitory concentration, while avoiding potential side effects of systemic administration.[1] Moreover, the minimum inhibitory concentration of bacteria that are within

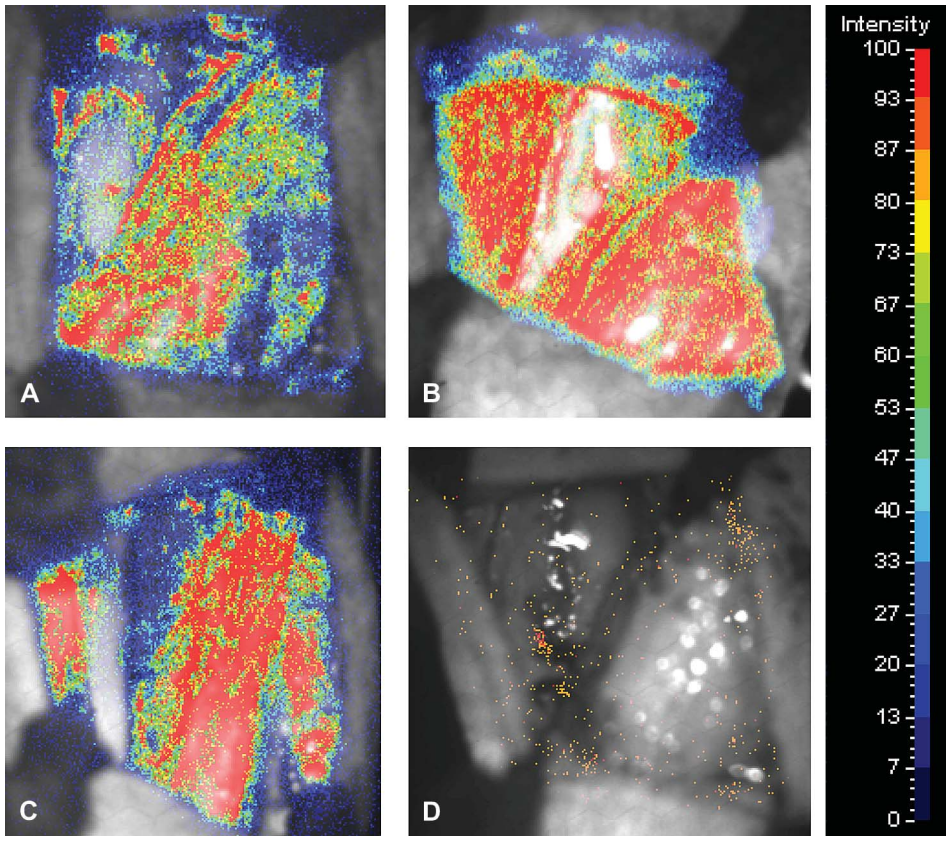

FIGURE 6. Representative imaging for Study II (wounds contaminated with *Staphylococcus aureus* [lux]): Control group images are shown on the top with the 6-hour predébridement and irrigation image (A) and 48-hour image (B). The vancomycin-loaded chitosan sponge group is shown on the bottom with the 6-hour predébridement and irrigation image (C) and 48-hour image (D).

a biofilm, which can form in as little as 3 hours, can be 1000 times higher than that of planktonic bacteria.[14] Therefore, systemic antibiotics may never be able to reach effective levels for the bacteria within a biofilm.

One of the more commonly used vehicles for local antibiotic delivery is with PMMA beads. Using this technique, antibiotic-impregnated PMMA beads are placed in the wound bed after adequate irrigation and débridement just before wound closure or coverage with a semipermeable membrane (bead pouch).[15] Use of antibiotic-impregnated PMMA beads has been shown to significantly reduce infection in extremity wounds[2]; however, an additional procedure is required for removal because they are not biodegradable. In addition to evoking a foreign body response, PMMA beads provide an attractive surface for biofilm-forming bacteria when no longer eluting antibiotics.[16] The chitosan sponges used in this study are biodegradable; therefore, local delivery of antibiotics can be performed in a single procedure without the need for surgical removal at a later point. Therefore, this degradable delivery system has an advantage over the cement beads because it can be used during definitive closure.

Antibiotic-impregnated calcium sulfate pellets have also been used clinically, and they degrade over time, thereby avoiding a second procedure for removal, similar to the chitosan sponge.[11,16] Both animal models and clinical studies have shown success with the use of antibiotic-impregnated calcium pellets in minimizing infection.[1,3,12] However, wounds treated with antibiotic-impregnated calcium sulfate pellets may develop sterile draining sinuses.[3] Although they usually heal on pellet resorption, they can mimic an infected draining sinus as a result of the osmotic effect of the chemical dissolution of the material into Ca^{2+} and SO^{2-} ions. In addition, unlike calcium sulfate pellets, chitosan-based implants have been shown to evoke only a minimal foreign body reaction with little or no fibrous encapsulation because the chitosan final breakdown products are saccharides.[17,18]

Chitosan has received a significant amount of attention recently for its potential use in wound healing, scaffolds, and drug delivery system applications.[6,19,20] In addition to its ability to promote hemostasis and wound healing, it has intrinsic antibacterial properties.[6,20] However, in vitro testing, including standard microbiologic assays (zone of inhibition and turbidity), demonstrated no bacterial inhibition of plain chitosan film and sponges before loading with antibiotics.[6,7] In an effort to conserve animals and in light of these in vitro data, chitosan sponges without antibiotics were not included in this study. The chitosan sponges used in this study were initially freeze-dried and stored in sterile peel packs. Immediately before placement in the wound, they were rehydrated by the surgeon with the antibiotic solution on the back table, a process that took less than 1 minute. Although only amikacin and vancomycin were tested in the present study, the chitosan sponges could be used with any water-soluble antibiotic allowing for broad-spectrum or culture-directed treatment; formulation changes can be made to tune both the degradation and elution kinetics to dissolve faster or slower.

Therefore, the chitosan sponge delivery system could potentially be used for many different clinical applications.

This study has several limitations. First, although the hindleg of a goat is not equivalent to the human leg, its relative size and subcutaneous nature allow for relevant preclinical in vivo testing of clinician-dependent interventions in a contaminated complex musculoskeletal wound model.[8–11] In addition, although previous in vivo testing demonstrated minimal foreign body response to chitosan,[18] histologic evaluation of wound specimens was not performed during this study. However, all wounds treated with the chitosan sponges did show subjective evidence of advanced wound healing compared with controls based on difficulty reopening the surgical incision.

In summary, the chitosan sponges used in this preliminary study were effective in delivering antibiotics locally and reducing bacteria within the wounds. In addition to being biocompatible and resorbable, the chitosan sponge delivery system can be loaded at the point of care with clinician-selected antibiotics, making this technology a potential drug delivery device of the future.

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